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# The transcriptional regulator, Arg82, is a hybrid kinase with both monophosphoinositol and diphosphoinositol polyphosphate synthase activity

Tong Zhang, James J. Caffrey, Stephen B. Shears*

*Inositide Signaling Section, Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA*

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**Abstract The *Arg82* gene of *Saccharomyces cerevisiae* encodes a transcriptional regulator that phosphorylates inositol 1,4,5-trisphosphate [Saiardi et al. (1999) Curr. Biol. 9, 1323–1326]. However, some controversy has surrounded the nature of the reaction products. We now show that Arg82 phosphorylates inositol 1,3,4,5-tetrakisphosphate to inositol pentakisphosphate, which is itself converted to two isomers of diphosphoinositol tetrakisphosphate, one of which has never previously been identified. One of the diphosphoinositol phosphates was further phosphorylated by a yeast cell lysate. We propose that Arg82 is an ancestral precursor of two distinct and specific enzyme families: inositol 1,4,5-trisphosphate kinases and diphosphoinositol polyphosphate synthases.** 

*Key words:* Inositol 1,3,4,5-tetrakisphosphate; Inositol polyphosphate multikinase; Arg82; Inositol pyrophosphate; Yeast

## 1. Introduction

The *Saccharomyces cerevisiae* gene, *Arg82*, encodes a 40 kDa pleiotropic transcriptional regulator [1–3]. Arg82 also associates with and stabilizes [4] Mcm1 (the product of the minichromosome maintenance gene), one of the eponymous members of the MADS (Mcm1, agamous, deficiens, serum response factor) box transcription factor family. Recently, this field of research took an unexpected new direction, with the discovery that Arg82 has inositol phosphate kinase activity [5,6].

However, controversy has quickly begun to engulf research into the molecular processes by which Arg82 links inositol phosphate turnover and transcriptional regulation. Initially, it was proposed that the inositol phosphate kinase activity of Arg82 played a direct role in the transcriptional process [5]. Later work suggested that the role of Arg82 is instead to facilitate the assembly of the multimeric transcriptional complex; in this model Arg82 is not one of the essential constituents of the final transcriptional unit [4,7,8]. Furthermore, a recent report presented direct evidence that inositol phosphate turnover can be dissociated from the processes by which Arg82 regulates the transcription of a group of genes that control arginine metabolism [9].

Research into Arg82 has been further confused by some dispute over its catalytic activity. While there is agreement that Arg82 phosphorylates Ins(1,4,5)$P_3$ to Ins(1,4,5,6)$P_4$, and then to Ins(1,3,4,5,6)$P_5$ [5,6,10], there are conflicting reports that Arg82 either does [6] or does not [5] phosphorylate Ins(1,3,4,5)$P_4$. This is an important point because, in addition to the Ins(1,4,5)$P_3$ 3-kinase reaction catalyzed by Arg82, there is a very active alternative pathway of Ins(1,3,4,5)$P_4$ synthesis in *S. cerevisiae* [10]. There is also a report that Arg82 phosphorylates Ins(1,4,5)$P_3$ up to Ins$P_6$, presumably through an Ins(1,3,4,5,6)$P_5$ kinase activity [6]. The ability of a single enzyme to synthesize Ins$P_6$ from Ins(1,4,5)$P_3$ was also attributed to a 41 kDa protein purified from *Schizosaccharomyces pombe* several years ago [11]. This protein therefore seems very similar to Arg82 in both size and catalytic activity. However, others have reported that Arg82 does not phosphorylate Ins(1,3,4,5,6)$P_5$ [5]. It seems essential that we come to some agreement as to the catalytic activity of Arg82, in order to simplify the difficulties we are encountering in deciphering its molecular roles in transcriptional regulation (see above).

The current study focuses on addressing this confusion over catalytic activity. We now show that Arg82 does indeed phosphorylate both Ins(1,3,4,5)$P_4$ and Ins(1,3,4,5,6)$P_5$. However, we make the surprising observation that Ins(1,3,4,5,6)$P_5$ is not converted to Ins$P_6$, as was originally thought [6], but instead it is a mixture of diphosphorylated inositol phosphates that are formed. We also conducted kinetic experiments to address the significance of this reaction. As well as resolving some controversy concerning the catalytic activity of Arg82, our new data provide new evidence in support of the close evolutionary relationship that exists between the enzymes of diphosphoinositol polyphosphate synthases, and the specific Ins(1,4,5)$P_3$ 3-kinases.

## 2. Materials and methods

Wild-type and the kcs1 'knock-out' yeasts and recombinant human type 1 diphosphoinositol polyphosphate phosphohydrolase (DIPP1), were obtained as described previously [6,12,13]. Recombinant Arg82 was expressed in *Escherichia coli* as previously described [6]. Yeast lysates were prepared as previously described [14]. Incubations of Arg82 were generally performed at 37°C in medium containing 20 mM HEPES (pH 7.2), 12 mM $MgSO_4$, 10 mM $Na_2ATP$, 20 mM phosphocreatine, 1 mM dithiothreitol, 1 mM EDTA, 360 U phosphocreatine kinase (Calbiochem) and 0.5 mg/ml bovine serum albu-

*Corresponding author. Fax: (1)-919-541 0559.
E-mail: shears@niehs.nih.gov

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min (BSA). Assays with yeast lysate were conducted in a similar manner, except that 10 mM NaF was included in the assay buffer, which slightly inhibits dephosphorylation of diphosphorylated inositol phosphates. Human DIPP1 was incubated in buffer containing 50 mM KCl, 50 mM HEPES (pH 7.2), 2 mM $MgSO_4$, 1 mM EDTA and 0.2 mg/ml BSA. Unless otherwise indicated, all reactions were conducted under first-order conditions with trace amounts of $^3$H-labeled substrates. Assays were quenched with PCA, and neutralized with $K_2CO_3$ and analyzed by high-performance liquid chromatography (HPLC) using a Partisphere SAX column as previously detailed [10]. For reasons of clarity, only selected portions of the HPLC chromatograms are shown; there were no significant levels of $^3$H-labeled products in fractions outside the range shown in our figures. All of the data that are shown in the current study are representative experiments, but they were repeated between three and five times with similar results.

[$^3$H]Ins(1,3,4,5)$P_4$ and [$^3$H]Ins$P_6$ were purchased from New England Nuclear. [$^3$H]Ins(1,3,4,5,6)$P_5$, PP-[$^3$H]Ins$P_4$ and [PP]$_2$-[$^3$H]Ins$P_3$ were prepared as previously described [12]. The PP-[$^3$H]Ins$P_4$ that was synthesized by Arg82 was desalted as detailed in an earlier study [13]. Non-radioactive Ins(1,4,5)$P_3$ and Ins(1,3,4,5,6)$P_5$ were purchased from CellSignals (Lexington, KY, USA).

## 3. Results and discussion

### 3.1. Phosphorylation of Ins(1,3,4,5)$P_4$ by Arg82

Ins(1,3,4,5)$P_4$ is actively synthesized in *S. cerevisiae* by both Arg82 and another unresolved enzyme [10]. Ins(1,3,4,5)$P_4$ has been reported not to be phosphorylated by Arg82 by York et al. [5]. While Snyder et al. [6] have stated the opposite, namely, that Ins(1,3,4,5)$P_4$ is phosphorylated by Arg82, the latter authors did not present any data describing these experiments, and so any reaction products were not identified. We therefore began this study with an HPLC analysis of the reactivity of Arg82 towards [$^3$H]Ins(1,3,4,5)$P_4$ (Fig. 1). Under our assay conditions, in as little as 10 min, almost all of the Ins(1,3,4,5)$P_4$ was phosphorylated; the product co-eluted exactly with standards of Ins(1,3,4,5,6)$P_5$ (Fig. 1). The only alternative Ins$P_5$ product, the 1,2,3,4,5 isomer, elutes approximately 5 min later than Ins(1,3,4,5,6)$P_5$ using our HPLC gradient [15]. Our assay conditions are a little different from those used by York et al. [5]. However, in further experiments, we also used their assay conditions (except that they monitored transfer of [$^{32}$P] from ATP to 10 μM non-radioactive Ins(1,3,4,5)$P_4$, whereas we used 10 μM [$^3$H]Ins(1,3,4,5)$P_4$); again, we found Ins(1,3,4,5)$P_4$ to be readily phosphorylated (data not shown).

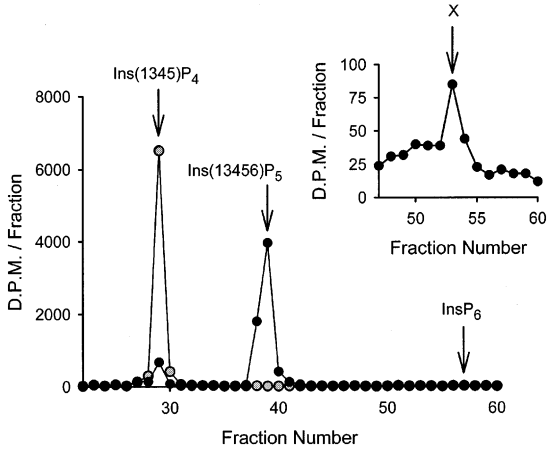


Fig. 1. Phosphorylation of Ins(1,3,4,5)$P_4$ by Arg82. Data in the main panel were obtained when approximately 7000 dpm of [$^3$H]Ins(1,3,4,5)$P_4$ was incubated in 100 μl assay buffer with 1.1 ng Arg82 for either 0 min (gray circles) or 10 min (black circles) as described in Section 2. Reactions were quenched, neutralized and analyzed by HPLC as described in Section 2. Arrows indicate the elution position of standards of [$^3$H]Ins(1,3,4,5)$P_4$, [$^3$H]Ins(1,3,4,5,6)$P_5$ and [$^3$H]Ins$P_6$. In the inset, reactions were allowed to proceed for 60 min, and small amounts of a novel product ('X') were observed.

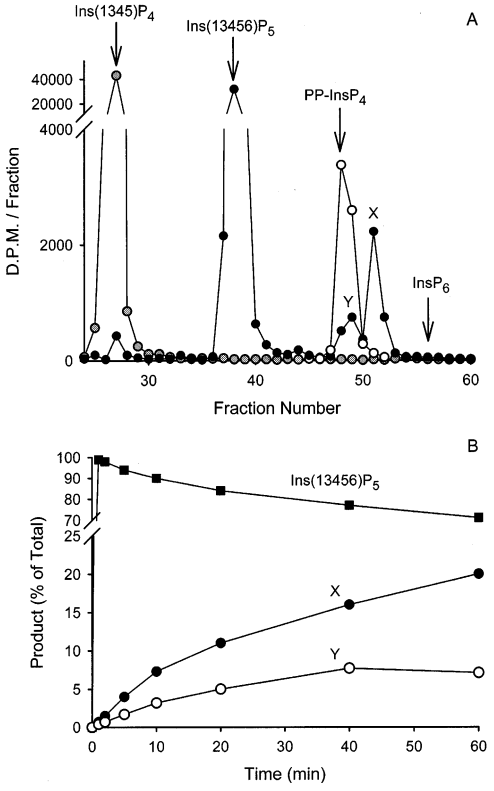


Fig. 2. Arg82 has diphosphoinositol polyphosphate synthase activity. A: Approximately 40 000 dpm [$^3$H]Ins(1,3,4,5)$P_4$ was incubated with 110 ng Arg82 for either 0 min (gray circles) or 10 min (black circles) as in Section 2. Reactions were quenched, neutralized and analyzed by HPLC as described in Section 2. Arrows indicate the elution position of standards of [$^3$H]Ins(1,3,4,5)$P_4$, [$^3$H]Ins(1,3,4,5,6)-$P_5$ and [$^3$H]Ins$P_6$. In a subsequent HPLC run (white circles), a standard of approximately 6000 dpm of [$^3$H]PP-Ins$P_4$ (see Section 2) was chromatographed. In (B), a time-course for these assays is depicted, showing the accumulation of Ins(1,3,4,5,6)$P_5$ (black squares), peak 'Y' (open circles) and peak 'X' (black circles).

Our study took an unexpected turn when we noted that, in reactions that were allowed to proceed for 60 min, a small amount of a novel $^3$H-labeled product was found to elute at 53 min (peak 'X' in the inset to Fig. 1). An earlier study proposed that Arg82 phosphorylates Ins(1,4,5)$P_3$ all the way to Ins$P_6$ [6]. However, peak 'X' is not Ins$P_6$, which eluted 4 min later (Fig. 1). A pH of 7.2 was optimum for the rate of accumulation of peak 'X', and addition to the assay buffer KCl (up to 150 mM) had no effect on the reaction rate (data not shown).

When larger quantities of [$^3$H]Ins(1,3,4,5)$P_4$ were used as substrate, and the concentration of Arg82 in the assays was increased 100-fold, the accumulation of peak 'X' could be more clearly monitored (Fig. 2A). Furthermore, it was now found that peak 'X' was preceded by an additional, smaller

peak (peak 'Y' in Fig. 2A; the ratio of peak 'X' to peak 'Y' was approximately 70:30). To identify the nature of these two peaks, we prepared a standard of genuine PP-[$^3$H]InsP$_4$ (Fig. 2A) by using the type 1 mammalian diphosphoinositol polyphosphate synthase (also known as the 'InsP$_6$ kinase') to phosphorylate [$^3$H]Ins(1,3,4,5,6)P$_5$ (see [12]). The resulting PP-[$^3$H]InsP$_4$ standard co-eluted exactly with peak 'Y' (Fig. 2A). Thus, peak 'Y' and peak 'X' are proposed to be two isomers of PP-[$^3$H]InsP$_4$, at least one of which (peak 'X') has not previously been identified.

Time-course experiments (Fig. 2B) indicated that Arg82 phosphorylated all of the Ins(1,3,4,5)P$_4$ to Ins(1,3,4,5,6)P$_5$ within 1 min, and only subsequently did either isomer of PP-[$^3$H]InsP$_4$ (peaks 'X' and 'Y') begin to accumulate. Peaks 'X' and 'Y' accounted for 25% of total products by the end of these experiments (Fig. 2B). No InsP$_6$ was formed. Thus, we propose that PP-InsP$_4$ is the product of Arg82 phosphorylation that Saiardi et al. [6] thought was InsP$_6$. The thin layer chromatography assays that the latter study used [6] would probably not distinguish PP-InsP$_4$ from InsP$_6$. This conclusion is also relevant to the catalytic activities of the apparent homolog of Arg82 that has been isolated from *S. pombe* [11]. The multifunctional inositol phosphate kinase activity of the latter protein included phosphorylation of Ins(1,3,4,5,6)P$_5$, and at that time, the product was thought to be InsP$_6$. Our data indicate that we should now reconsider if the actual reaction product might have been PP-InsP$_4$.

While this manuscript was under review, Saiardi et al. [16] published their cloning and analysis of a putative human homolog of Arg82, named mammalian inositol polyphosphate multikinase (mIPMK). They show in their report that Ins(1,4,5)P$_3$ is phosphorylated to a novel compound that, upon HPLC, eluted prior to InsP$_6$. They proposed the novel product was a diphosphorylated inositol phosphate, but they did not perform any further analyses to confirm this. They also indicated that Arg82 did not phosphorylate Ins(1,3,4,5,6)P$_5$ [16]. Our demonstration that Ins(1,3,4,5,6)P$_5$ is phosphorylated by Arg82 therefore shows its functional relationship with mIPMK is closer than previously suspected.

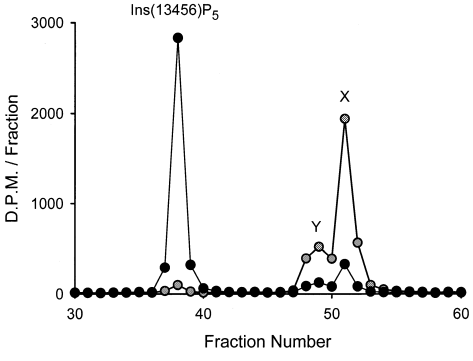


Fig. 3. DIPP-catalyzed hydrolysis of the PP-InsP$_4$ formed by Arg82. Approximately 3000 dpm of peak 'X' and 'Y' that were formed by Arg82 (Fig. 2) were incubated in 50 μl assay buffer with 2 μg DIPP for either 0 min (gray circles) or 60 min (black circles) as described in Section 2. Reactions were quenched, neutralized and analyzed by HPLC as described in Section 2.

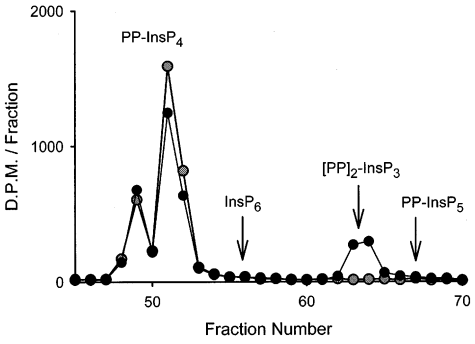


Fig. 4. Phosphorylation of PP-InsP$_4$ by yeast lysate. Approximately 3000 dpm of [$^3$H]PP-InsP$_4$ that was formed by Arg82 was incubated with 0.3 μg of a lysate of *S. cerevisiae* in 200 μl assay buffer for either 0 min (gray circles) or 60 min (black circles) as described in Section 2. Reactions were quenched, neutralized and analyzed by HPLC as described in Section 2. Arrows depict the elution positions of [$^3$H]InsP$_6$, PP-[$^3$H]InsP$_5$ and [PP]$_2$-InsP$_3$, determined with standards in parallel HPLC runs.

### 3.2. Confirmation that Arg82 acts as a diphosphoinositol polyphosphate synthase

Peaks 'X' and 'Y' were further analyzed using, as a diagnostic tool, DIPP1 [13]. DIPP1 specifically removes the β-phosphate from diphosphate groups placed around the inositol ring [13]. DIPP1 does not exhibit any phosphomonoesterase activity [13]. The putative PP-[$^3$H]InsP$_4$ isomers that comprise peaks 'Y' and 'X' in Fig. 2A were prepared in bulk, HPLC-purified, desalted and incubated with DIPP1. Between 80 and 85% of both PP-[$^3$H]InsP$_4$ isomers were dephosphorylated back to Ins(1,3,4,5,6)P$_5$ (Fig. 3); in longer experiments the reactions went to completion (data not shown). Not only does this experiment confirm the presence of a diphosphate group in peaks 'X' and 'Y', but it also shows the latter could not be PP-InsP$_3$, which would have been converted by DIPP1 to InsP$_4$. Henceforth, we refer to peaks 'X' and 'Y' as two isomers of PP-InsP$_4$.

To understand more of the physiological kinase activities of Arg82, we examined the kinetic properties of the monophosphoinositol and diphosphoinositol synthase activities of this enzyme. In three experiments, we found the mean $K_m$ value for Ins(1,4,5)P$_3$ was 15.3 μM, and the $V_{max}$ was 6272 nmol/mg protein/min. The levels of Ins(1,4,5)P$_3$ in *S. cerevisiae* have never been directly measured, but radiolabeling experiments indicate it comprises $<2\%$ of the level of InsP$_6$ [10], which itself probably has an upper solubility limit in cells of approximately 100 μM [17]. With Ins(1,4,5)P$_3$ levels therefore unlikely to be greatly in excess of 1 μM in *S. cerevisiae*, we can anticipate that Ins(1,4,5)P$_3$ will be phosphorylated under first-order conditions in vivo. The same will also be true of Ins(1,3,4,5,6)P$_5$, since its steady-state cellular levels are similar to those of Ins(1,4,5)P$_3$ [10] and, furthermore, we found the $K_m$ for Ins(1,3,4,5,6)P$_5$ phosphorylation by Arg82 to be 62 μM, with a $V_{max}$ of 4.9 nmol/mg protein/min. These in vitro data suggest Ins(1,3,4,5,6)P$_5$ is a relatively poor substrate for Arg82, so it is somewhat paradoxical that relatively large amounts of PP-InsP$_4$ have been observed in *S. cerevisiae* under some conditions [18]. Moreover, diphosphoinositol polyphosphates are typically dephosphorylated very rapidly in intact cells [19]. Furthermore, elimination from *S. cerevisiae* of the InsP$_6$ kinase does not eradicate diphosphorylated ino-

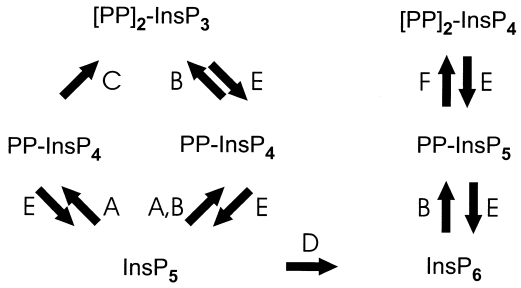


Fig. 5. Turnover of diphosphoinositol polyphosphates in *S. cerevisiae*. The schematic shows the synthesis and metabolism of diphosphoinositol polyphosphates in yeast, which include reactions catalyzed by Arg82 (A), kcs1 [6,12] (B), an unidentified kinase in cell lysates (C, see Fig. 4), the Ins(1,3,4,5,6)$P_5$ 2-kinase [24] (D), YOR163w, the *S. cerevisiae* homolog of DIPP [25] (E), and the yeast PP-Ins$P_5$ kinase [21] (F). Note that two different isomers of PP-Ins$P_4$ are formed.

sitol phosphates from cells [20], so there must be alternative pathways for their synthesis. Thus, it is possible that the rate of substrate utilization by Arg82 is different in vivo from what the in vitro data indicate. This would not be unexpected if inositol phosphate kinase activity is closely tied to the participation of Arg82 in the transcriptional process [5].

### 3.3. Synthesis of diphosphoinositol polyphosphates by *S. cerevisiae*

To gain insight into the metabolic fate of PP-Ins$P_4$, we incubated both isomers (see above) with yeast cell lysates (Fig. 4). We found that PP-Ins$P_4$ was further phosphorylated to a product that co-eluted with a standard of [PP]$_2$-Ins$P_3$ (Fig. 4). The latter is a doubly diphosphorylated product of Ins(1,3,4,5,6)$P_5$ (Fig. 5). It is also notable that it was the later-eluting of the two PP-Ins$P_4$ isomers that was primarily phosphorylated (Fig. 4). Kcs1 has previously been shown to synthesize [PP]$_2$-Ins$P_3$ from PP-Ins$P_4$ [12], and may be in part responsible for the reactions seen in Fig. 4. However, we also observed PP-Ins$P_4$ phosphorylation in cell lysates made from yeast in which the kcs1 gene had been eliminated (data not shown). Presumably, yet another kinase remains to be characterized (Fig. 5).

Fig. 5 provides an updated picture of the pathway of diphosphoinositol polyphosphate turnover in yeast. In both intact cells and cell lysates, it is difficult to accurately ascertain the rate of synthesis of diphosphoinositol polyphosphates, because they are degraded so rapidly [19]. In mammalian cells, it has proved analytically helpful that the phosphatases are fluoride-sensitive [13,19]. However, in yeast cell lysates, considerable dephosphorylation of diphosphoinositol polyphosphates occurs even in the presence of fluoride [21]. An important challenge for the future will be to ascertain the relative rates of fluxes through the individual metabolic reactions shown in Fig. 5.

### 3.4. Evolutionary relationships between inositol phosphate kinases

Arg82 is the first inositol phosphate kinase shown to exhibit both monophosphoinositol and diphosphoinositol polyphosphate synthase activities. Whether or not turnover of diphosphorylated inositol polyphosphates is as complex in higher eukaryotes as it is in yeast (Fig. 5) may be an important area of future research.

An inositol phosphate binding domain has been identified in Arg82, at the center of which is a $Px_3DxKxG$ motif [6]. This motif is also seen in mammalian Ins(1,4,5)$P_3$ 3-kinases [6], but these are highly specific enzymes that are not known to phosphorylate any other naturally occurring inositol phosphates, neither do they form diphosphorylated inositol phosphates (see [22] for a review). Conversely, the same motif is also present in diphosphoinositol polyphosphate synthases, but these enzymes do not produce monophosphoinositol polyphosphates [6,12,23]. The unusual hybrid catalytic activity of Arg82 suggests it might be an ancestral precursor of both the diphosphoinositol polyphosphate synthases and the Ins(1,4,5)$P_4$ 3-kinases. This new idea that the latter two groups of proteins diverged from a common ancestor, contrasts with a recent proposal that the Ins(1,4,5)$P_3$ 3-kinases evolved from the diphosphoinositol polyphosphate synthases [23]. Not only do our studies with Arg82 open up new ideas concerning the evolution of inositol phosphate signaling, they can also act as a foundation upon which further structural work can decipher the catalytic mechanisms of monophosphoinositol and diphosphoinositol polyphosphate synthesis.

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## References

[1] Bercy, J., Dubois, E. and Messenguy, F. (1987) Gene 55, 277–285.
[2] Dubois, E. and Messenguy, F. (1994) Mol. Gen. Genet. 243, 315–324.
[3] Qui, H.F., Dubois, E., Bröen, P. and Messenguy, F. (1990) Mol. Gen. Genet. 222, 192–200.
[4] El Bakkoury, M., Dubois, E. and Messenguy, F. (2000) Mol. Microbiol. 35, 15–31.
[5] Odom, A.R., Stahlberg, A., Wente, S.R. and York, J.D. (2000) Science 287, 2026–2029.
[6] Saiardi, A., Erdjument-Bromage, H., Snowman, A., Tempst, P. and Snyder, S.H. (1999) Curr. Biol. 9, 1323–1326.
[7] Amar, N., Messenguy, F., El Bakkoury, M. and Dubois, E. (2000) Mol. Cell. Biol. 20, 2087–2097.
[8] Shears, S.B. (2000) Bioessays 22, 786–789.
[9] Dubois, E., Dewaste, V., Erneux, C. and Messenguy, F. (2000) FEBS Lett. 486, 300–304.
[10] Saiardi, A., Caffrey, J.J., Snyder, S.H. and Shears, S.B. (2000) FEBS Lett. 468, 28–32.
[11] Ongusaha, P.P., Hughes, P.J., Davey, J. and Michell, R.H. (1998) Biochem. J. 335, 671–679.
[12] Saiardi, A., Caffrey, J.J., Snyder, S.H. and Shears, S.B. (2000) J. Biol. Chem. 275, 24686–24692.
[13] Safrany, S.T., Caffrey, J.J., Yang, X., Bembenek, M.E., Moyer, M.B., Burkhart, W.A. and Shears, S.B. (1998) EMBO J. 17, 6599–6607.
[14] Wuestehube, L.J. and Schekman, R. (1992) Methods Enzymol. 219, 124–128.
[15] Glennon, M.C. and Shears, S.B. (1993) Biochem. J. 293, 583–590.
[16] Saiardi, A., Nagata, E., Luo, H.R., Sawa, A., Luo, X., Snowman, A.M. and Snyder, S.H. (2001) Proc. Natl. Acad. Sci. USA 98, 2306–2311.
[17] Irvine, R.F., Moor, R.M., Pollock, W.K., Smith, P.M. and Wreggett, K.A. (1988) Philos. Trans. R. Soc. Lond. (Biol.) 320, 281–298.
[18] Ives, E.B., Nichols, J., Wente, S.R. and York, J.D. (2000) J. Biol. Chem. 275, 36575–36583.

[19] Menniti, F.S., Miller, R.N., Putney Jr., J.W. and Shears, S.B. (1993) J. Biol. Chem. 268, 3850–3856.
[20] Caffrey, J.J., Safrany, S.T., Yang, X. and Shears, S.B. (2000) J. Biol. Chem. 275, 12730–12736.
[21] Ali, N., Duden, R., Bembenek, M.E. and Shears, S.B. (1995) Biochem. J. 310, 279–284.
[22] Shears, S.B. (1998) Biochim. Biophys. Acta 1436, 49–67.
[23] Schell, M.J., Letcher, A.J., Brearley, C.A., Biber, J., Murer, H. and Irvine, R.F. (1999) FEBS Lett. 461, 169–172.
[24] York, J.D., Odom, A.R., Murphy, R., Ives, E.B. and Wente, S.R. (1999) Science 285, 96–100.
[25] Safrany, S.T., Ingram, S.W., Cartwright, J.L., Falck, J.R., McLennan, A.G., Barnes, L.D. and Shears, S.B. (1999) J. Biol. Chem. 274, 21735–21740.